artment of Agriculture Agricultural Research Service June 1992

Agricultural Research

Noxious Pest Meets New Nemesis

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ARS' Commitment to Feeding People

The U.S. Department of Agriculture's ultimate mandate has always been to ensure that the nation has a safe, adequate food supply.

We cannot forget that food does not originate on a cardboard tray or in a plastic container. Food does not just happen. It has to be grown by someone, packaged, and brought to a market to be purchased for your dinner table.

But "safe and adequate" goes far beyond simply having enough bushels of wheat, tons of tomatoes, or gallons of milk available to meet consumer demands. It also means producing a variety of foods that, collectively, provide all the nutrients essential for a balanced diet.

Congress recognized that need in 1893 when it first appropriated $10,000 "to enable the Secretary of Agriculture to investigate and report upon the nutritive value of various articles and commodities used for human food with special suggestion of full, wholesome, and edible rations, less wasteful and more economical than those in common use."

That put USDA squarely in the middle of nutrition research. And, as USDA's chief scientific agency, ARS bears primary responsibility for pursuing this mandated research.

The first need is for precise knowledge of what the human body needs for optimal growth and health. Thanks in part to ARS research, we now know that age, sex, and other factors make a decided difference in people's nutritional needs.

But a hundred years ago, when modern nutrition research was in its infancy, it was commonly held that minimum nutrient requirements were all the same as long as the subject was human. Actually, those early requirements were predicated on meeting the nutrient needs of adult males.

ARS research has come a long way toward refining that early and erroneous presumption.

In the 1950's, ARS accumulated much of the data for women's dietary requirements. Now, ARS is focusing on the very specific nutrient needs of people at both extremes of the age spectrum.

The elderly are the single fastest growing segment of our population, and we need to know what they should be eating to maintain optimal health and quality of life.

At the ARS Human Nutrition Research Center on Aging at Tufts University in Boston, many new findings suggest that modest dietary modifications may greatly improve the health status of the elderly. For example, researchers have found that vitamin E and other antioxidants enhance older people's resistance to disease by stimulating their immune systems. Increased vitamin C may protect them against cataract formation.

The special nutrient needs of pregnant and lactating women, and of preborn, newborn, and young children are intensively studied at the ARS Children's Nutrition Research Center at the Baylor School of Medicine in Houston, Texas.

And at the ARS Western Human Nutrition Research Center in San Francisco, California, scientists are looking at the American diet as a whole, to see if particular populations or regions might be lacking essential nutrients. Such knowledge is important for making widespread dietary improvements.

With ever more sophisticated methods and instrumentation, ARS continues to reach for more precise measurements and understandings of what food components are essential to human health.

Scientists at the ARS Human Nutrition Research Center in Grand Forks, North Dakota, recently found that the human diet needs to include 3 to 5 milligrams of boron per day. Adequate boron not only contributes to building healthy bones and maintaining proper brain function, but may also help prevent osteoporosis.

At the ARS Beltsville Human Nutrition Research Center in Maryland, our scientists have now discovered that too little of the mineral chromium in the diet may lead to many cases of adult-onset diabetes. Chromium studies at the Beltsville center date back to 1969, when researchers there first recognized chromium's role as an essential element.

But knowing what nutrients we need is only one side of the coin. Its opposite side is finding out what foods contain what levels of those essential nutrients. ARS has a special mandate to develop the standards and methods for determining the exact nutrient composition of foods. The results of such studies help provide guidance for improving crops' nutrient values.

Back in the 1940's, the first big project at ARS' U.S. Plant, Soil, and Nutrition Research Laboratory in Ithaca, New York, was a study of fertilizer's effect on the carotene content of tomatoes. Carotene is a precursor to vitamin A.

Today, an ARS researcher is busy breeding a new tomato that could easily be in the same range of vitamin A content as sweetpotatoes, ounce for ounce, or provide nearly half the vitamin A of the average carrot.

Other research projects to improve the nutritional quality of foods stretch from developing soybeans with higher levels of sulfur-containing amino acids, making soy protein more complete, to developing leaner meats through genetic and management techniques.

So, after nearly 100 years of effort, progress continues toward a more complete understanding of human dietary needs. And ARS continues in its long-term commitment to feeding people...better.

R.D. Plowman
Administrator
Agricultural Research Service

Agricultural Research

Cover: Costly crop pests like this cotton bollworm may soon encounter a new biological control—the celery looper virus—being tested by the Agricultural Research Service and a commercial laboratory. Photo by Scott Bauer. (K-4695-1)

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Vol. 40, No. 6
June 1992

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Agricultural Research is published monthly by the Agricultural Research Service, U.S. Department of Agriculture, Washington, DC 20250-2350.

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Subscription requests should be placed with New Orders, Superintendent of Documents, P.O. Box 371954, Pittsburgh, PA 15250-7954. Please see back cover for order form.

Address magazine inquiries or comments to: The Editor, Information Staff, Room 316, Bldg. 005, 10300 Baltimore Ave., Beltsville Agricultural Research Center-West, Beltsville, MD 20705.

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Microbe Blows Those Hornworms Away

Tobacco hornworm caterpillar (about three times life-size). (K-990-8)

Growers and gardeners may get new weapon to combat insect pests.

Tired of being hassled by giant hornworm caterpillars that always seem to show up on your favorite tomato plants?

Help is on the way.

ARS scientists in California, Arizona, and Mississippi are honing a new weapon that, within a few years, might stave off this and other unwelcome pests that worry backyard gardeners and commercial growers alike.

Known as the celery looper virus, this naturally occurring microbe might be purified, then packaged and sold as an environmentally friendly viral insecticide, or biopesticide.

This could happen within the next 5 years, says ARS entomologist Patrick V. Vail at Fresno, California. He says the virus kills susceptible insects within a few days after they eat it.

Viral insecticides aren't new. But the celery looper virus stands out from others because of an impressively broad array of destructive insects that it kills.

Pests that succumb to the microbe, for instance, include not only its namesake, the pale-green celery looper worm, but also cabbage loopers, tomato and tobacco hornworms, cotton bollworms (a.k.a. corn earworm and tomato fruitworm), and pink bollworms. Also susceptible: tobacco budworms that eat tobacco, cotton, tomatoes, garden peas, and other crops; southwestern and European corn borers; codling moths that are especially damaging to apples, pears, and walnuts; and diamondback moths and imported cabbageworms that are pests of cabbage, broccoli, and other crops.

That's according to lab tests by the codiscoverers of the virus, Donald L.

JACK DYKINGA

Entomologist Pat Vail examines a dying cotton bollworm infected with the celery looper virus. (K-4578-17)

Hostetter, who is with ARS at Kimberly, Idaho, and ARS retiree Ben Puttler, now with the University of Missouri. The team made entomological history in 1990 by becoming the first to win a U.S. patent for this kind of microbe, known as a baculovirus.

According to Hostetter, no other currently known natural or genetically engineered baculovirus infects as many key crop-destroying insect species or kills them as quickly at low doses. Here's an example of just how costly some of those insects are: Cotton bollworms rob U.S. growers of about $1.5 billion each year, when damage losses and the cost of control measures are combined.

At strategic sites in the Cotton Belt, bollworms are among the pests entomologist Vail and ARS colleagues Thomas J. Henneberry at Phoenix, Arizona, and M. Randy Bell at Stoneville, Mississippi, are targeting this year.

In nature, bollworms and many of the other insect pests that transform into moths might accidentally encounter looper virus particles while nibbling on leaves or cotton bolls.

Once ingested, the virus commandeers the machinery inside the insect's cells, causing them to churn out billions of copies of the virus.

As the virus proliferates, the caterpillar loses its appetite. When it dies from the infection, it literally falls apart, disintegrating into liquid. The infectious mess oozes onto leaves or dribbles to the ground. The next insect that chances on these deadly particles may start the cycle all over again.

The virus is almost always lethal: Once infected, insects that are susceptible can't recover. That's not the case with another biopesticide, *Bacillus thuringiensis,* or Bt, produced by a naturally occurring microorganism. "Insects that eat leaves, stems, or bolls covered with Bt may crawl away and recover," Vail says.

A disadvantage of Bt and another popular pesticide, synthetic pyrethroids, is that some formerly susceptible insects may become resistant. Similar resistance hasn't yet surfaced in preliminary tests of the celery looper virus, according to Vail.

Too, the broad assemblage of insects that the celery looper virus attacks may give it a competitive advantage over another older biopesticide, the *Helicoverpa* (formerly *Heliothis*) *zea* virus. Preliminary lab and field tests by Vail indicate that

JACK DYKINGA

Samples of the celery looper virus are prepared for analysis by biological technician Darlene Hoffman. (K-4577-9)

Cabbage looper caterpillar. (K-2827-2)

the celery looper virus does about as well as the *H. zea* virus in killing tobacco budworms and cotton bollworms. But the looper virus also goes after other cotton enemies that the *H. zea* virus can't harm, such as the cabbage looper and pink bollworm.

"That's important," notes Vail, "because a mixture of pests may occur in the same field of cotton at the same time." Depending on what region a cotton plant is growing in, it could at any time be beset by these four—and many more, including yellowstriped and beet armyworms and soybean loopers.

The celery looper virus infects all of these species. In fact, the looper virus appears so promising that Sandoz Agro, Inc., former marketer of the older virus, now holds an exclusive research and development agreement with ARS to test the newer one.

Last year the company tried the looper virus on cabbage and broccoli fields at five farms on the east and west coasts. This year, Sandoz will test it on these crops plus tomatoes, alfalfa, and cotton.

At the company's Palo Alto, California, laboratories, Sandoz scientists seek to streamline production of the virus. They currently rely on a slurry furnished by ARS' Randy Bell, who uses a laboratory colony of tobacco budworms as factories for the virus.

Healthy budworm caterpillars are about 1-1/2 inches long and light to dark green, or even brown, with narrow, pale stripes running lengthwise along their backs. The doomed laboratory caterpillars, however, die soon after they reach full size. Their corpses are ground up, then placed in a centrifuge that whirls the liquid at high speeds, separating virus particles from unwanted contaminants. This partially purified virus is freeze-dried to a fine, grey powder or stored in liquid.

Sandoz researchers aim to replace this slow and costly manufacturing process with an approach that's worked with other microbes—namely, to replicate the virus in laboratory colonies of insect cells kept alive in test tubes. Such tissue culturing is cleaner and potentially cheaper and simpler.

TIM McCABE

The Palo Alto scientists also hope to boost the microbe's virulence and quicken its action. One possibility: Augment the virus with natural toxin genes borrowed from other organisms. "The added genes would work only inside insects that the virus infects and not in other creatures," explains Nikolai Van Beek, a senior scientist at Sandoz.

Researchers elsewhere have already genetically engineered insect viruses. These high-tech viruses, however, would likely take longer to garner regulatory approval from the U.S. Environmental Protection Agency.

Although four virus-based insecticides are currently OK'd by that agency for use in the United States, none is now marketed commercially. Competition from synthetic pyrethroids, plus the slow-acting nature of the viruses, likely combined to keep these and other viral insecticides on the sidelines.

That may change.

Sandoz' backing for the looper virus, and a new agreement between two other corporations—Du Pont and Crop Genetics International—to market other viral insecticides, signal new interest in these natural organisms.

What's more, ARS and American Cyanamid have negotiated a research and development agreement to explore compounds that—in laboratory tests—increase the deadliness of certain viruses 100- to 1,000-fold. These new ventures, says researcher Hostetter, may speed the transformation of the celery looper microorganism and other promising insect viruses, as well, into marketable products.—By **Marcia Wood,** ARS.

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Potato Chippers See Orange

Could there be a novel snack on the horizon?

A new potato with orange flesh and a creamy white or red skin could be an unusual addition to our current selections of this common vegetable.

Potatoes with flesh "about the color of a cantaloupe" have never been reported, according to geneticist Charles R. Brown, who discovered them in one of his test plots.

The egg-sized spuds turned up in experimental crosses of potatoes originally collected in the Andes.

The new orange tubers shouldn't be confused with sweetpotatoes or yams, which are each classified in botanical families that are different from those of regular potatoes.

Brown says they do have an aftertaste "somewhat like a sweet–potato but without the sweetness," and a texture that's a tad mealy. "Personally, I like them, but I must admit I look for the novel when it comes to potato flavor."

Curiously, the new potatoes contain very little beta carotene—a naturally occurring pigment that imparts an orange hue to carrots, sweetpotatoes, and yams. Our bodies convert beta carotene into vitamin A.

The potatoes do, however, contain high levels of other, related carotenoids called zeaxanthin and lutein. The two compounds are xanthophylls—also present in many other plants. Corn, squash, and citrus contain the compounds, as do green leafy vegetables. We also see them in the reds and yellows of autumn leaves.

As yet, the nutritional value of zeaxanthin and lutein is unknown. But Brown says further research may reveal their potential as antioxidants. Such compounds (beta carotene and vitamins C and E are good examples) are thought to retard aging and possibly protect against cancer.

The zeaxanthin trait is controlled by a single gene, Brown says. "We think it's a different form of the gene that controls yellow flesh color."

Potatoes with yellow flesh are well known—especially in Europe, where they're more common than white ones. Consumers may also occasionally spot the odd, purplish-blue-fleshed spuds in grocery stores. Their color comes from still another plant pigment, called anthocyanin, found in the potato's skin.

Potato breeders from several snack food companies have requested samples of the new orange potatoes.

"We're interested in them because their novelty might boost consumer appeal," says Bob Hoopes, a breeder geneticist with Frito-Lay, who is based in Rhinelander, Wisconsin.

Hoopes crossed several of the orange-fleshed tubers with varieties Frito-Lay currently uses for potato chips. Most weren't as bright as the orange parents, but had deep-yellow-colored flesh instead.

"We've made new crosses this year to try and recover the orange color," Hoopes says.

Potatoes were first cultivated by South American Indians about 8,000 years ago. Spanish explorers introduced potatoes to Europe in the 16th century and immigrants from Ireland and Scotland later brought them to North America. Today, they're one of our most popular vegetables. The average American eats 129 pounds of potatoes each year—about one-third of a pound per day.—By **Julie Corliss,** ARS.

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SCOTT BAUER

Orange-fleshed "white" potato. (K-4648-14)

Vitamin C Shortage Undermines Antioxidant Defense System

Imagine eating bran muffins, cereal, and low-fat milk for breakfast; a tuna sandwich and chocolate ice cream for lunch; then chicken teriyaki, rice, rolls, and shortbread cookies for dinner.

What's wrong with these meals?

Because they don't include any fruits or vegetables, your body would eventually run out of—among other things—vitamin C.

These menus and others like them were specially designed for a 13-week ARS study of the human need for vitamin C. And one of the most important findings, according to ARS research chemist Robert A. Jacob, was that skimping on vitamin C lowers the body's concentration of glutathione. "Glutathione," says Jacob, "is a powerful component of your antioxidant defense system."

Antioxidants defend the body from damaging byproducts of natural internal chemical reactions that go on all the time. Without protective antioxidants, the risk of heart disease, cancer, or inflammatory diseases like arthritis would increase.

Vitamin C is itself a hard-working antioxidant. So are vitamin E, carotenes (compounds your body uses to make vitamin A), the essential mineral selenium, and some enzymes.

This investigation is apparently the first of its kind to link a lowered intake of vitamin C to a decrease in glutathione. "This could happen to anyone who consistently eats less than the 60-milligram Recommended Dietary Allowance of vitamin C," notes Jacob.

Americans most likely to be getting less than the RDA include smokers (cigarette smoke destroys the vitamin) and snackers who prefer foods high in fats and carbohydrates to fruits and vegetables. A small bowl of strawberries, a serving of kale leaves, or a few broccoli florets easily provide the RDA. Other foods especially rich in C include brussels sprouts, green peppers, collards, cauliflower, oranges, lemons, and grapefruit.

A 250-milligram dose of vitamin C—a level also used in the experiment—about equals that furnished in two servings of fruit and three of vegetables, as recommended in the USDA and National Cancer Institute guidelines for healthful eating.

Jacob conducted the study with eight volunteers, men aged 25 to 43, at the ARS Western Human Nutrition Research Center, San Francisco, California. His research colleagues were Susanne M. Henning, Jian Z. Zhang, Ralph W. McKee, and Marian E. Swendseid from the University of California, Los Angeles.

PERRY RECH

Chemist Robert Jacob checks results from an automated analyzer that is used to determine nutrient levels in blood. (K-3527-13)

The decline in glutathione showed up within 9 weeks after the volunteers began consuming only 5 to 20 milligrams a day of vitamin C. That's less than one-third the RDA. During this low-C phase of the study, glutathione levels in blood samples fell by 50 percent. "We were surprised to see glutathione change so noticeably in such a short time," Jacob says. Levels doubled when Jacob later boosted volunteers' vitamin C intake to 60 or 250 mg.

The researchers also noted two other signs of oxidative damage. Volunteers' blood levels of NAD and NADP—two niacin-derived enzymes—increased when vitamin C levels dropped. "That indicates stress to your antioxidant mechanism," Jacob explains.

Further, quantities of oxidated guanine, another telltale indicator of oxidative damage, about doubled in sperm cells when volunteers went from the study's highest level of vitamin C—250 mg—to the low of 5 to 20 mg. Oxidated guanine levels decreased by 36 percent, however, when Jacob hiked vitamin C intake to 60 or 250 mg a day. Cancer researcher Bruce Ames and colleagues at the University of California, Berkeley, collaborated with Jacob in this work.

"Sperm may be particularly sensitive to oxidation and need vitamin C's protection more than other cells. Perhaps that's why vitamin C levels in semen are about eight times higher than in blood."

To control each volunteer's vitamin C intake throughout the study, only foods that have little if any vitamin C—such as meats, breads, rice, pasta, cookies, cake, and low-fat milk—were served. For part of the time, soft drinks that accompanied each meal were spiked with measured amounts of powdered vitamin C.

Jacob admits that although the meals were tasty, they lacked the attractive colors of fresh produce. Ironically, volunteers had few complaints. "It's sort of a sad commentary," he says, "that no one particularly minded going without fruits or vegetables."—By **Marcia Wood,** ARS.

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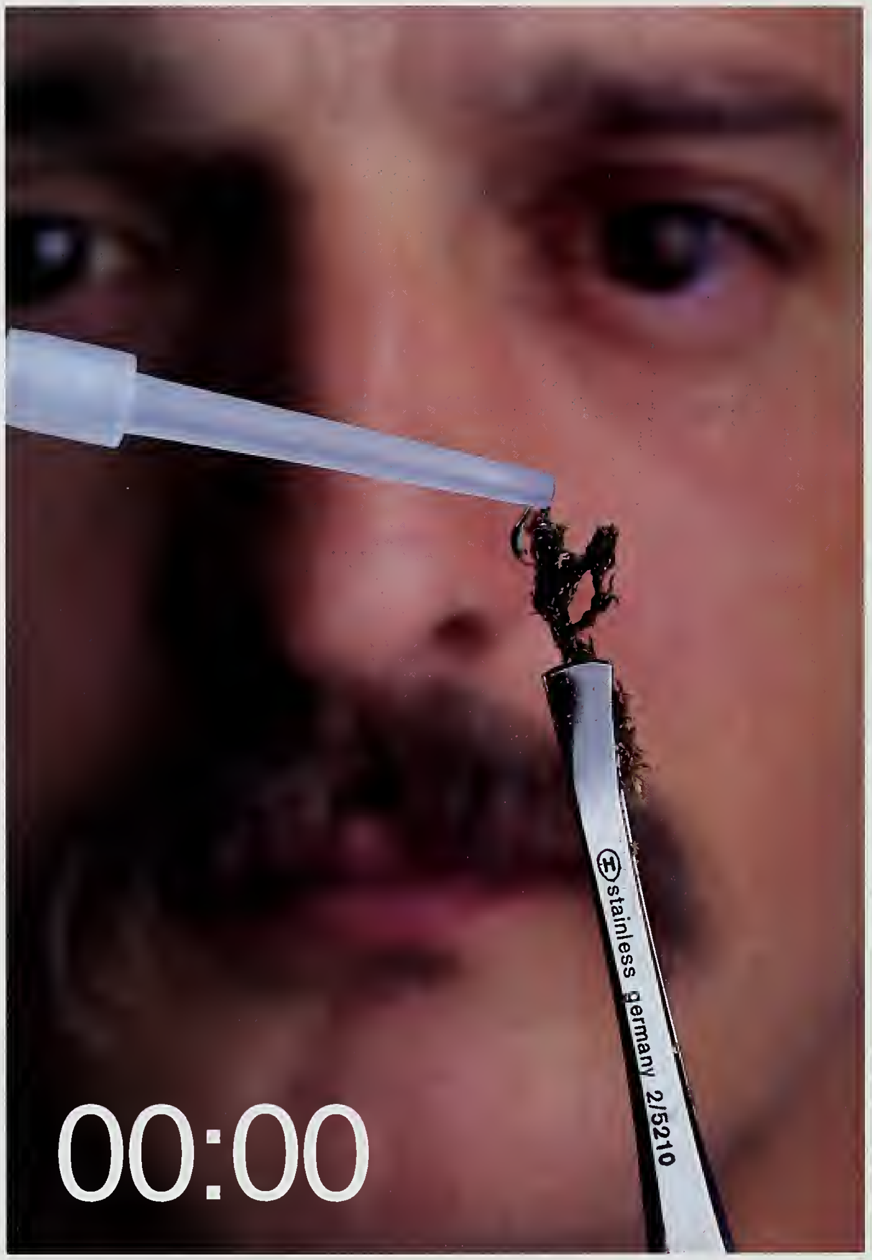
Miracle Moss: Add Water and Watch It Grow

Moss may have genes to make lawns and crops truly drought-tolerant

JACK DYKINGA



With a few drops of water, molecular biologist Mel Oliver demonstrates star moss' amazing recovery from drought. Moss begins to show green leaves within 30 seconds of rewetting. (K-4671-13,14, 15)

A moss found throughout the United States may hold a gene that will give drought tolerance a new meaning for U.S. crops.

Agricultural Research Service molecular biologist Mel Oliver collects the moss from mountainous U.S. regions. He believes the key to improving economically important crops lies in the vast and relatively untapped gene pools of noncrop plants such as the lowly star moss, *Tortula ruralis.*

It grows throughout the world but is particularly abundant in North American wilderness areas.

Star moss' quick recovery from drought can be easily demonstrated. As soon as a few drops of water are poured on the dry moss, what once seemed a brown Brillo pad becomes a lush green mass of individual branches with starlike needles.

Viewed through an electron microscope, the dried moss shows massive cell damage. "And yet it somehow repairs most of this damage within minutes," Oliver says.

In the long term, Oliver envisions lawns, rangelands, and pastures that could do the same. "We're talking about using genetic engineering to create a grass that can approach the capability of the star moss to completely dry up, turn brown, and recover quickly when it rains," he says.

But Oliver is no mere visionary. It's taken long hours at ARS' Cropping Systems Laboratory in Lubbock, Texas, to put him on the trail of the responsible moss genes.

Oliver reasons that clues to the drought-repair gene begin with those proteins that increase during the recovery period, after the moss has dried out and been remoistened. With two-dimensional electrophoresis—a protein separation technique that uses electrical current and an acrylamide gel—he has found 74 proteins that increase significantly within 2 hours of rewetting.

To find out which genes make these proteins, Oliver extracts RNA genetic material from moss tissue during its critical drought-recovery period. He uses this RNA to make

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DNA copies of all the genes active during this stage.

RNA and DNA are the biological vehicles for transmitting genetic or hereditary characteristics and for building proteins in plants and animals.

Oliver plans to use the DNA copies as probes to locate the specific star moss genes responsible for its quick drought recovery. But to do this, he must first isolate all the individual moss genes.

Then he will separate each DNA copy of interest into its two exactly matching—complementary—strands and radioactively label one of them. The labeled strands will be put into solutions to interact with separated, fixed, unlabeled strands of the star moss genes. The DNA copies will rewrap themselves around only their exact genetic counterparts. This will mark the locations of the genes responsible for making each of the 74 proteins found so far during the recovery of star moss from drought.

Oliver hopes that only a few gene clusters encode for the proteins involved in the repair process, because that will make it easier to transfer the genes into crops.

"We're one of a few plant laboratories looking at the gene pool of a wild organism," Oliver says. "It shows why it's so important to protect the genetic variation of plants and animals in remote parts of the world. In star moss alone, we might find genes that could make crops truly drought-tolerant, allowing them to survive severe desert-type drought and not just the mild moisture shortages crops are currently bred to survive."

Oliver is pleased that what was once an academic interest to him while teaching at the University of Calgary in Alberta, Canada, may now change forever the meaning of crop drought tolerance.—By **Don Comis**, ARS.

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Probing for Stress-Tolerant Plants

Fiber optic probes are not only aiding doctors in diagnosing people; they're also helping agronomists diagnose stress in plants.

Decapitation from grazing or harvesting, over-exposure to nitrate from manure or commercial fertilizer, soil temperatures too low or too high, or drought—all are enough to make soybeans, alfalfa, clover, and other legumes less efficient nitrogen fixers. But, just like people, some plants may endure more adversity than others with little or no loss in productivity.

R. Ford Denison, an ARS plant physiologist, has built an apparatus that can quickly identify the toughest plants.

If he wants to check their response to grazing, he cuts the plant a half inch above ground. If he's checking the effects of drought or other stresses, he doesn't need to take so drastic a step.

In either case, he brushes just enough dirt away to expose one of the root nodules containing nitrogen-fixing bacteria. Next he places a probe so that two attached optical fibers touch the nodule, one on each side.

High-intensity red and infrared light pass through one fiber, illuminating the nodule. The ratio of red to infrared light returning through the second fiber to a photodetector is used to calculate the concentration of oxygen in the nodule.

Denison says the nodules have to maintain an optimal amount, because too little or too much oxygen would be harmful.

He explains that the apparatus he designed has confirmed in the field what has been reported recently from his and other laboratories, including that of Thomas R. Sinclair, an ARS plant physiologist in Gainesville, Florida: Reduction in nitrogen fixation is caused by oxygen starvation of the bacteria inside the legume nodule. "Stress causes the nodule to admit less oxygen," he says, "and the bacteria's ability to supply nitrogen from the air to the plants slows down."

SUSAN BOYER

Light passing through 1-mm fiber optic probes (touching the root nodule) measures the plant's response to stress.

An adequate oxygen supply in the nodule of a detopped plant is a sign that that particular plant tolerates stress well. Denison presented comparison data for alfalfa and birdsfoot trefoil in the March 1992 issue of *Plant Physiology.*

Just like people, some plants may endure more adversity than others with little or no loss of productivity.

Says Dennison, "In addition to improving our understanding of plant responses to stress, we see the instrument as a way plant breeders can do something about the oxygen deprivation/stress connection. They can use it in selecting legumes in the greenhouse or field that are superior nitrogen-fixers in the face of stress.

There have been other attempts to monitor the oxygen content of legume nodules, but all were practical only in a laboratory. "This is the first portable instrument for field testing," notes Denison.

He and a Canadian collaborator, David Layzell, have a patent pending on the instrument. A U.S. company, Morgan Scientific, Inc., is developing a commercial version.

Denison says he can test each nodule in less than 5 minutes, not counting the time required to find it.—By **Don Comis,** ARS.

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Bruising in Tomatoes

It's a complex puzzle that may have a genetic solution.

You've been thinking about it all the way home—that huge, mouth-watering tomato on the windowsill, so perfect for that cool summer salad. You pick up the tomato and inwardly groan as you feel the soft spot that means a bruise and, possibly, a spoiled fruit.

We take the bruising and consequent spoiling of fruits and vegetables as a simple, annoying fact of life. But in fact, "there is nothing simple about it. Bruising sets in motion a complex set of genes. It is a process that we don't yet fully understand," says molecular biologist Barbara L. Parsons, who works at the Beltsville Agricultural Research Center in Maryland.

"If we can find all the genes involved in the bruising process, we may be able to change the genetic makeup of plants so they can resist the spoilage caused by bruising," says Parsons.

When a fruit or vegetable is bruised or wounded, certain genes are turned on while others are turned off.

"In our studies, we've isolated three new genes that are involved with the wounding process," says Parsons.

"Ethylene—a natural plant hormone that causes aging and is produced during bruising—normally turns on each of these genes. However, one of the genes we isolated was turned off during bruising, presenting us with another puzzle to solve," says Parsons.

"This gene also produces a protein rich in glycine that may impart plasticity to the cell wall. Our goal is to decode the sequence of events regulating all of the genes that are involved in bruising."

Millions of dollars are lost annually as a result of bruising during harvesting, shipping, and storage. For one crop alone—tomatoes—30 to 40 percent are bruised and consequently spoil, says plant physiologist Autar K. Mattoo, who is in charge of ARS' Plant Molecular Biology Laboratory in Beltsville, Maryland.

Tomatoes are shipped green and do not produce ethylene, the natural ripening agent, for a few days. This ensures that they won't ripen until they reach the supermarket.

If these same tomatoes are bruised, ripening is accelerated by the ethylene that is turned on by bruising. The initial production of ethylene causes more to be produced. "It is a self-perpetuating process—the hormone stimulates the gene to produce more and more ethylene—causing premature ripening," says Mattoo.

"To add insult to injury," says Mattoo. "there are many wound pathogens—bacteria and fungi—that attack the fruit only when injury occurs. That is why bruising that beautiful tomato results in a rotten spot or a completely spoiled fruit."—By **Vince Mazzola,** ARS.

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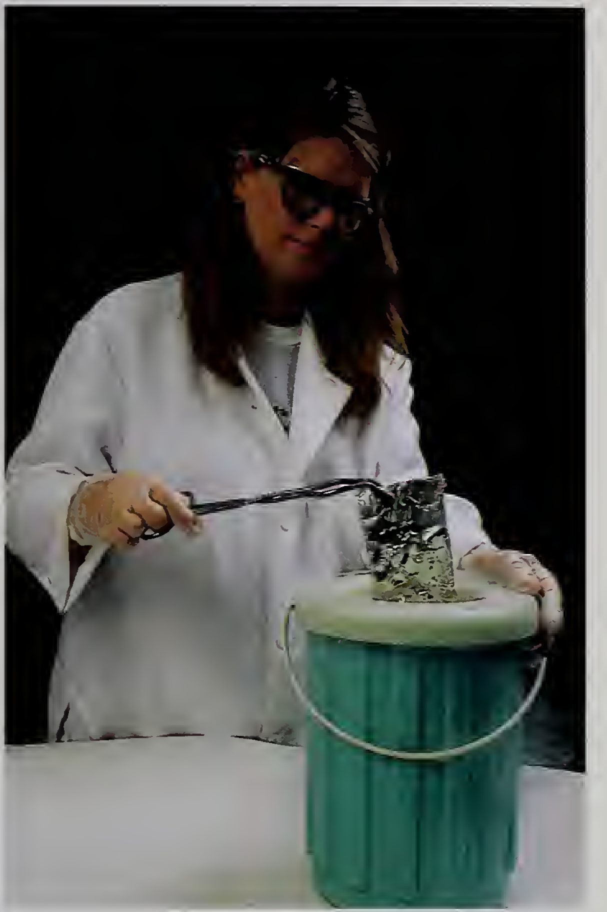
SCOTT BAUER



Molecular biologist Barbara Parsons lowers foil-wrapped injured tomato tissue into liquid nitrogen in preparation for RNA isolation. (K-4172-1)

SCOTT BAUER

Tomatoes are readied for bruise-damage analysis. (K-4172-5)

ARS Hosts Institute for Higher Learning—About Alfalfa

To Aimeé Crago, what science is all about is "the feeling I had, when I finally realized something had been discovered and the information would be useful."

"Useful" might prove to be an understatement. The work she performed may help keep millions of dollars from being stolen from alfalfa producers by a plant disease.

During the last two summers, Crago, of Metairie, Louisiana, helped the Agricultural Research Service rate alfalfa plants for resistance to one of alfalfa's worst diseases. Yield loss from anthracnose, caused by the fungus, *Colletotrichum trifolii,* can easily reach $200 million nationally in years when severe epidemics strike.

In the summer of 1990, Crago spent 5 weeks working at an ARS lab near Washington, D.C. This nonpaying position was part of her internship with the Research Science Institute.

RSI is a program sponsored by the Center for Excellence in Education, a private nonprofit organization based in McLean, Virginia. CEE was created in 1984 by the late Admiral Hyman G. Rickover, generally considered the father of the U.S. Navy's nuclear submarine fleet.

In the summer of 1991, after earning her high-school diploma at Mount Carmel Academy in New Orleans, Crago returned to ARS as a summer employee. She also served as counselor for several "Rickoids," as RSI interns are known.

During both summers, Crago helped plant pathologist Nichole O'Neill in the Soybean and Alfalfa Research Laboratory, part of the agency's Agricultural Research Center in Beltsville, Maryland.

Crago's work contributed to the rating of more than 2,000 strains of alfalfa through O'Neill's research program. The strains included commercial varieties and breeding lines as well as plants grown from seed stored in an ARS germplasm collection.

To rank alfalfa for anthracnose resistance, Crago grew thousands of seedlings in lab growth chambers. She sprayed them with a liquid solution of fungus spores and recorded how many survived. O'Neill compiled the results for use by commercial firms and by scientists in Pullman, Washington, at an ARS germplasm repository for alfalfa, beans, and many other plant species.

SCOTT BAUER

Summer employee Aimeé Crago stains an alfalfa cotyledon for microscopic examination. (K-4264-8)

Until now, O'Neill points out, researchers and industry haven't had this kind of information in a standard format.

And while many alfalfa strains resist race 1 of the fungus, Crago's experiments confirmed that two breeding lines had rare, high resistance to race 2, which is a problem mainly in the Southeast.

A breeder plans to release one of the lines next year as a new, anthracnose-resistant commercial variety, O'Neill notes.

"Furthermore," she adds, "we're interested in the mechanism behind race 2 resistance and suspect it may differ from that in plants that withstand race 1. If we can identify the mechanism and the genes controlling it, we might be able to insert the genes into alfalfa plants. Then industry can develop new, highly resistant commercial varieties for farmers.

"It was wonderful for Aimeé to see that her work here was useful," O'Neill notes. "But it's easy for scientists to forget that even the things we see as routine—such as how to design an experiment so we can have confidence in the results—are valuable new experiences for students."

One of Crago's reports on her ARS experiments helped her win honors as a semifinalist in the Westinghouse Talent Search for 1990.

Thirteen RSI interns ran a variety of experiments at Beltsville last summer. Their scientist mentors were recruited by chemist James Saunders, a colleague of O'Neill's. Two dozen other D.C.-area agencies and private firms supplied mentors for the rest of the 103 RSI interns.

"Admiral Rickover founded the institute to help our best students and teachers keep the United States competitive in science and technology," says CEE president Joann DiGennaro.

RSI's features make it fairly unique, she adds. "It's free, provides long-term followup and tracking, and selects interns—nearly all entering high-school seniors—entirely by merit."

In Washington, Rickoids live in a dormitory at George Washington University. A week of academic courses is followed by 4 weeks of internship and a final week during which students make oral and written presentations on the work they've done.

But vacation is not slighted: On weekends the interns go camping or visit the region's amusement parks, beaches, and other attractions.

"Two years ago," Crago says, "I didn't even know what alfalfa plants looked like, and I was apprehensive about spending my vacation studying them. But by the end of that first summer, my experience had proven to me that a career in research was what I wanted eventually."

Now attending Tulane University in New Orleans, she's considering a future in medical biochemical research.

A year ago, when she was honored at the White House as one of 141 Presidential Scholars, President Bush quoted Crago in his address. She had written earlier that "to be the person one wants to be, one needs to have a knowledge of all the people one could be, and education provides this knowledge."—By **Jim De Quattro,** ARS.

Nichole O'Neill is in the USDA-ARS Soybean and Alfalfa Research Laboratory, Beltsville Agricultural Research Center, 10300 Baltimore Ave., Beltsville, MD 20705-2350. Phone (301) 504-5331.

For further information on the Research Science Institute contact Joann P. DiGennaro, President, Center for Excellence in Education, 7710 Old Springhouse Rd., McLean, Virgina 22102. Phone (703) 448-9062. ◆

SCOTT BAUER

Students Peter Khalifah (top) and Jeremy Vander Weele work on genetic research at ARS' Soybean and Alfalfa Research Laboratory in Beltsville, Maryland. (K-4266-14)

Mysteries of Ripening

We're making progress in understanding this natural process.

SCOTT BAUER

Low concentrations of [illegible] by Bernard Priem and [illegible]onia Yuno [illegible] tomatoes to ripen, while higher concen[illegible] inhibit ripening. (K-4654-19)

Senescence: synonym, old age. The state of being old; the process of aging.

Though we most commonly think of aging in human terms, it is a normal progression in all living things. And it follows a similar course: Ultimately, cells lose their rigidity and physical structures go soft.

ARS plant physiologist Kenneth C. Gross is actively seeking ways to slow down the softening that occurs with aging in fruits and vegetables.

Decreased marketability from this problem may cause the loss of up to half of all produce harvested in the United States, costing about $5 billion a year.

Successfully reducing postharvest losses, Gross says, will depend on our ability to understand what controls ripening and softening and then to genetically alter the produce or modify the postharvest environment.

In seeking answers, Gross and colleagues at the Horticultural Crops Quality Laboratory in Beltsville, Maryland, have made some startling discoveries.

"Contrary to accepted theory, plant cell wall formation doesn't stop when fruits reach maturity," says Gross. "It actually increases when they ripen. It's like there's a sudden burst of energy—a beginning rather than an ending."

But the new cell walls are probably different in composition—and more easily softened—than those in immature fruits.

For over 50 years it was thought that fruit softening was caused by an enzyme, polygalacturonase (PG), breaking down pectins in cell walls. The theory was accepted because PG is active during ripening, the amount of PG increases during the process, and PG breaks down cell wall structure in laboratory tests.

"No one ever proved this theory; it has just always been accepted," Gross says. "But look at strawberries and apples. Neither contain PG; yet both soften considerably." And when active PG has been inserted into mutant fruits that don't ripen, they still don't soften.

SCOTT BAUER

Research associate Ian DeVeau homogenizes tomatoes for further analysis of chemical changes involved in ripening. (K-4656-1)

So, if PG isn't responsible for fruit softening, then what is? Is there a way that science can change this natural process to ensure that the very thing that makes fruit appealing doesn't destroy its storability?

"We want to control softening so we can increase storage life and yet maintain high quality," Gross says. "And we're considering several possibilities."

Ian DeVeau, a plant physiologist working with Gross, has purified a new enzyme from an avocado extract. The enzyme has not yet been identified but may be one that is involved in softening.

"We've tested it and know that it does break down pectin," Gross says. "DeVeau is now trying to determine exactly what this enzyme is."

Meanwhile, a discovery by plant physiologist Cindy Tong, now at the University of Minnesota, is helping to shed light on the mystery of the ripening process. Tong found a specific cell wall component that exists only in mature green tomatoes, not ripe ones. When injected with this substance, nonripening mutant tomatoes ripen partially.

Similar substances, called N-glycans, were purified by ARS research associate Bernard Priem, who discovered that low concentrations stimulate tomatoes to ripen, while higher concentrations inhibit the process.

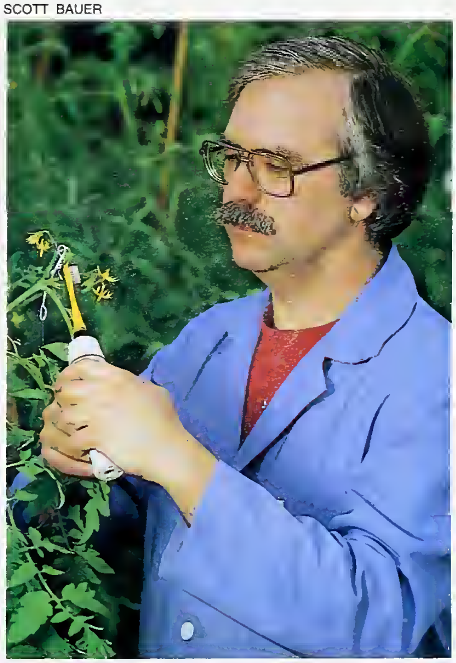
"Tong found that this substance is there only when needed, not before or after," says Gross.

Since N-glycans are part of the cell wall, they may be released from cell wall proteins as a signal in the ripening process. Gross and colleagues are looking for a cell receptor that might be sensitive to that signal.

"These results suggest new ideas that must be considered if the veil surrounding the mysteries of fruit ripening and softening is to be lifted," Gross says.—By **Doris Stanley,** ARS.

Kenneth C. Gross is at the USDA-ARS Horticultural Crops Quality Laboratory, Bldg. 002, 10300 Baltimore Ave., Beltsville, MD 20705-2350. Phone (301) 504-6128. ◆

SCOTT BAUER



Lab technician Norman Livsey hand-pollinates tomato flowers so their precise age at harvest can be determined. (K-4655-20)

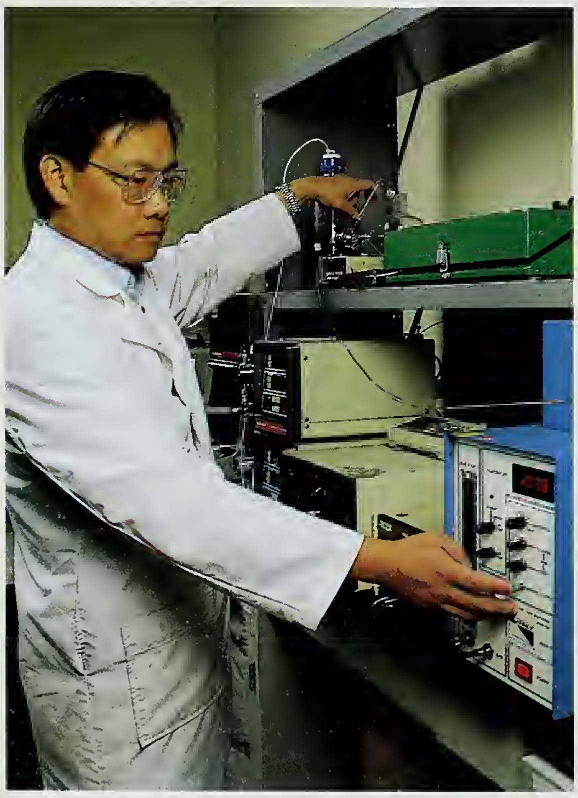
Cocoa Butter From Cottonseed Oil

It may be possible one day to nibble on a chocolate bar and narrow the foreign trade gap at the same time.

That's because Agricultural Research Service scientists have come up with a domestic substitute for cocoa butter, the primary ingredient in chocolate, baked goods, and a variety of other food and nonfood products. George Abraham, a chemical engineer, and M. K. Chang, a biochemist, both at the ARS Southern Regional Research Center in New Orleans, Louisiana, found a way to enzymatically make a cocoa-butter-like product from cottonseed oil and high-oleic acid sunflower oil.

SCOTT BAUER

SCOTT BAUER



Chemist Min-Kun Chang analyzes a cocoa-butterlike fat made from cottonseed oil and high-oleic acid sunflower oil. (K-4476-1)

Above: Chemical engineer George Abraham uses a differential scanning calorimeter to determine the melting characteristics of a cocoa butter substitute. (K-4465-20)

Right: A sample of cocoa butter substitute is prepared for melting- point analysis. (K-4465-1)

SCOTT BAUER

KEITH WELLER

Substitute cocoa butter can replace that made from real cocoa beans. (K-4637-13)

Cocoa butter is derived from the bean of the cacao plant.

"It's an expensive fat with a fluctuating price," says Abraham, who is in the SRRC's Food and Feed Processing Research Unit.

As there are no domestic sources, the United States shops for cocoa butter around the world. Malaysia and Brazil are the primary suppliers, meeting nearly half the annual U.S. needs.

According to the U.S. Department of Commerce, imports from all sources in the first 2 months of 1992 were about 19 million kilograms (42 million pounds) valued at nearly $58 million. The previous year, cocoa butter imports totaled about 93 million kilograms (205 million pounds) worth $279 million.

Abraham's product can replace pure cocoa butter in about 90 percent of its uses, he says. Molecules from cottonseed oil and sunflower oil are mixed in a reaction container with a commercially available enzyme derived from fungi. The enzymes cause a reaction that rearranges fatty acids in the oils, resulting in a mixture with many of the chemical and physical properties of natural cocoa butter.

"The reaction takes place at 150°F," Abraham says. "There are similar ways to make a cocoa-butter-like product that don't involve the use of enzymes, but they also don't produce as good a product." Other enzyme processes proposed in the past have been more complicated and used nondomestic oils as starting materials.

After the conversion occurs, the mixture passes through two further steps to separate the converted material from unconverted material. In the first of these two separation steps, both the converted and unconverted material enter a tank and are mixed with acetone solvent. The mixture is cooled to room temperature.

Unconverted cottonseed oil is drawn from the first tank and sent back to the reactor. The cocoa butter product and unconverted sunflower oil then flow to a second tank and are cooled to 40°F. The cocoa butter is recovered from the second tank.

That leaves a mixture of unconverted sunflower oil and acetone solvent, which is sent from the tank to an evaporator that recovers the acetone solvent. The unconverted sunflower oil is heated and returned to the reactor where the process initially began.

Says Abraham, "This process allows continuous isolation of the cocoa-butter-like product with continuous separation and recycling of unconverted material." —By **Bruce Kinzel,** ARS Information.

George Abraham is at the USDA-ARS Food and Feed Processing Research Unit, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124. Phone (504) 286-4339. ◆

KEITH WELLER

A new substitute can replace natural cocoa butter in about 90 percent of the products made with cocoa butter. (K-4638-13)

Corn Pest Logs Thousands of Frequent Flyer Miles

Corn growers, beware! The moths that are parents of the familiar black cutworm appear to be fly-by-night insects that originate in the South but migrate to midwestern cornfields.

The insects may travel at 60 to 70 miles per hour some 1,000 to 2,000 feet up, riding strong southerly winds ahead of cold fronts.

Black cutworm larvae are an intermittent anxiety for corn growers. In some years, the roving parents produce few offspring; the cutworms cause little damage and go unnoticed. But in other years, crops may be decimated by plant-chewing larvae.

BRUCE FRITZ

At Ankeny, Iowa, entomologist William Showers notes the nightly capture of black cutworm moths in a sex pheromone-baited trap. (K-4679-11)

Therein lies a job for scientists: to learn enough about biology and insect migration patterns to predict when trouble is likely to occur and to devise economical control strategies.

ARS entomologist William B. Showers at Ankeny, Iowa, and his colleagues have inferred details about the cutworm's travel itinerary after taking a close look at the pollen the moths carried.

After examining moths of night-flying black cutworm, *Agrotis ipsilon,* retrieved from sex-pheromone-baited traps in late April, it was clear the insects had flown a long distance. Still sticking to the moths' mouth parts were pollen grains from purple-flowering woody legumes such as Calliandra and Texas ebony *(Pithecellobium)* that grow not less than a 1,000 miles to the south.

That discovery proved for the first time that in the wild, black cutworm moths have a taste for travel.

From studies of laboratory-reared insects, the scientists had already determined that mother moths could migrate from the Texas and Louisiana Gulf Coast to Missouri, Kansas, and Iowa. The lab moths—also caught in pheromone traps—were marked by a telltale reddish cast caused by a fat-soluble red dye that had been added to their diet.

The dyed moths made the trip from either Crowley, Louisiana, or College Station, Texas, to Iowa within a matter of 3 or 4 nights.

As more is learned about the insect and its life cycle, scientists may become able to not only predict outbreaks but also prevent them at the source—somewhere farther south. But first, a lot of questions about the migratory cycle need to be answered, says Thomas W. Sappington, an Iowa State University entomologist who works with Showers.

Could there be genes in some individuals within black cutworm populations that cause them not to migrate? Do moths that fly south from the Midwest in the fall stop in Texas to produce another generation that flies to Mexico? How do environmental influences such as day length, temperature, and crowding affect migration?

Sappington is studying the migratory potential of moths reared in outdoor cages or in indoor insectaries and then tethered by glue to a flight mill in an environmental chamber where they get a workout. When light and temperatures simulate evenings of an April or perhaps October day, 1-to-5-day-old moths speed around the flight mill's 2-meter circumference past an electronic eye while a computer counts the revolutions. Sappington finds that 3-day-olds begin long-duration flights earlier—about 2 hours after sunset—than the other moths and that they fly about 6-1/2 hours.

In another flight-mill study, Sappington observed that female moths laden with fertilized eggs were almost as likely to make migratory-length flights and to fly almost as far as unmated females.

The observation, which supports a belief that most migrating females arrive in the Midwest already mated, was enlightening but not surprising, he says. In field studies in Ohio and Iowa, newly arrived mated females had been caught in light traps, but scientists were unsure when the mating took place. Had the matings occurred before a local flight to the trap or before or during migration?

The tentative answer—before or during migration—is being used to refine predictions of how soon cutworm larvae develop after the moths' arrival in an area.

In one set of experiments, Sappington set up wire-mesh "honeymoon"

BRUCE FRITZ

BRUCE FRITZ

BRUCE FRITZ

This black cutworm moth, tethered in a laboratory experiment, demonstrates the flying prowess which enables its kind to migrate from Texas to Iowa in just 3 or 4 nights. (K-4682-1, 2, 3)

cages where he placed pairs of the male and female moths. There, for two nights, they dined at a cotton ball soaked in honey-water and beer, and most moths successfully mated.

On the third night, it was time for the moths to take spins on the flight mill. Mated and unmated moths of both sexes had about the same tendency to engage in long-term flight.

Mated females began their long flights about 3 hours later in the evening than unmated females, but Sappington concluded that matedness doesn't seriously affect moths' ability to make long flights. The mated females flew just as fast and for only 2 hours less.

The experiments showed for the first time that in the springtime migratory period, young moths' hormones did not suppress reproductive maturation or sexual activity as migration approached, as happens in some other insects. However, in autumn this lack of suppression might not be true. To find out, scientists are conducting further laboratory and field studies.

Seemingly, the reproductive systems of black cutworm grandchildren of the spring migrators shut down during southward autumn migration time, thus preventing egg laying until the trip is completed. A possible explanation is that in autumn, northerly winds are usually at lower altitudes and have less velocity than the southerly low-level jets of spring. The flights south might take several days longer.

Computer Versus Cutworm

The researchers are combining this knowledge of black cutworms' migration from overwintering sites in the South with other discoveries to help crop consultants and farmers better time their field scouting to coincide with likely damaging outbreaks.

The Iowa Extension Service is using a computerized forecasting model

developed by a team of meteorologists, climatologists, economists, and entomologists. The scientists are refining the model in a 3-year pilot research program, now in its second year.

An earlier model put together by meteorologist Michael D. McCorcle, formerly of Iowa State University, described the migration of adult moths and forecast where, when, and in what concentrations the moths would be found in the Midwest. Then, climatologist S. Elwynn Taylor of the university and ARS' Showers developed a model to predict the development of larvae, when to expect first damage, and the likely extent of damage to a crop in areas of infestation. The model requires information relating to the moth's arrival, soil temperatures, and hatching of larvae from the moth's eggs.

To refine data input to the black cutworm forecasting model, entomology graduate student James J. McNutt, along with other research team members including economist Arne E. Hallem, began checking on the model's accuracy in 1991. The refinements may help improve prediction accuracy and let farmers make the most cost-effective management decisions.

For example, a historical analysis of weather patterns and predictions of weather that consistently cause late planting of a crop could justify applying an insecticide to a field before an expected black cutworm outbreak.

As McNutt monitored tilled and untilled fields with different planting dates within a 50-mile radius of Ames, he found that the model best predicted cutworm damage early in the season. In four fields, immigrant moths arriving in late April produced progeny that predictably destroyed about 5 percent of one-to-two-leaf seedlings of corn planted in early May. No-till fields provided a better environment for egg laying, larval survival, and seedling destruction.

Photographed at midnight, a hungry black cutworm larva topples a corn stalk.

Cutworm Forecast Joins the Weather News

"This year we're conducting a public test on delivering cutworm damage forecasts through television," says S. Elwynn Taylor of Iowa State University.

At a push of a button on a remote control, he says, TV sets equipped to receive World Text closed captions show the cutworm predictions, along with weather forecasts and information on gypsy moths and agricultural pests, while the audio portion of normal programming continues from a commercial station in Ames.

The closed captions may be expanded to include many more types of information of interest to urban and rural central Iowans.

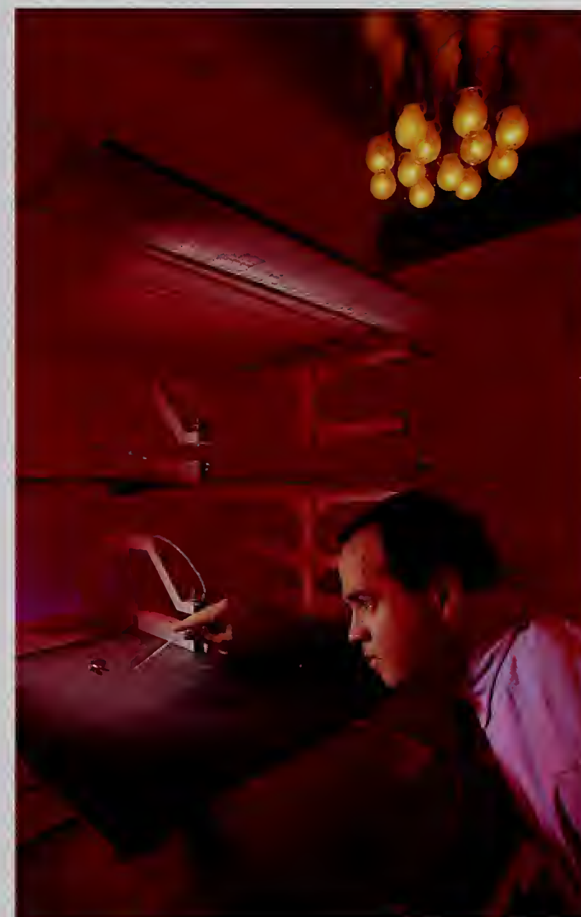
BRUCE FRITZ

Laboratory technician Delores Gunnarson observes black cutworms in an outdoor insectary at Ankeny, Iowa. (K-4683-1)

BRUCE FRITZ

In April, research assistant James McNutt surveys weeds for black cutworm larvae on an Iowa farm. (K-4680-1)

BRUCE FRITZ



A black cutworm moth gets a tethered-flight workout while research associate Thomas Sappington watches. (K-4681-1)

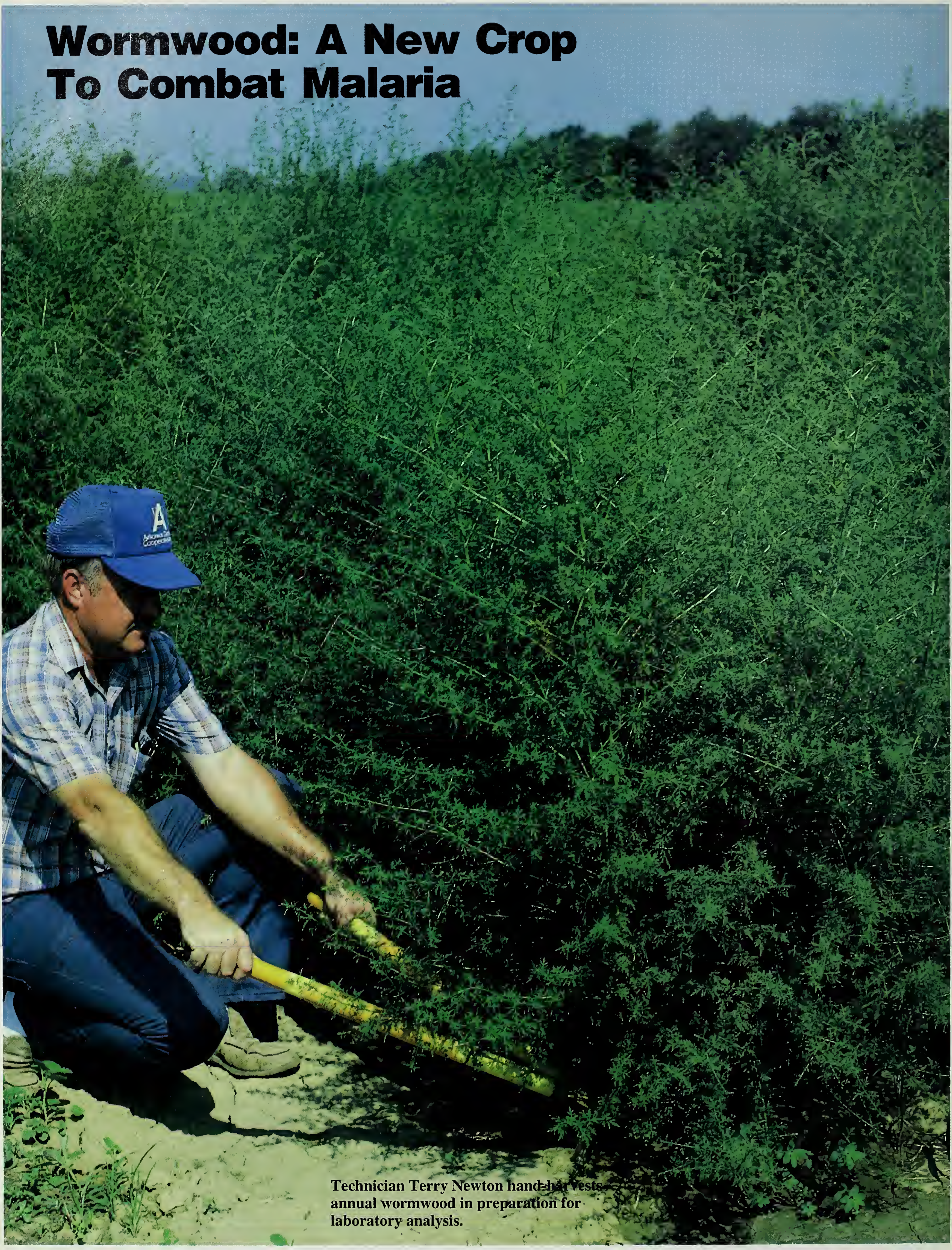
Weed management is an important part of minimizing black cutworm damage. Showers says destroying weeds at least 8 days before corn planting may be a good idea. Moths lay their eggs in fields that are weedy before corn is planted. But removing weeds soon after corn planting could be disadvantageous: Larvae with no weeds to feed on will turn to devouring corn seedlings.

When a black cutworm hatch is imminent, the research team has found that timely applications of insecticides best prevent outbreaks if the insecticides are not tilled into the soil. Insecticides used in the study included fenvalerate and permethrin, a synthetic chemical related to naturally occurring chemicals in chrysanthemum plants.—By **Ben Hardin,** ARS.

William B. Showers is in the USDA-ARS Corn Insects Research Unit, Ankeny Research Farm, Box 45B, Ankeny, IA 50021. Phone (515) 964-6664. ◆

CHARLES BRYSON

Wormwood: A New Crop To Combat Malaria



Technician Terry Newton hand-harvests annual wormwood in preparation for laboratory analysis.

Charles T. Bryson is a botanist—not a medical doctor—yet his work may eventually save millions of lives.

Bryson and colleagues at the ARS Southern Weed Science Laboratory at Stoneville, Mississippi, were asked by the University of Mississippi to develop a weed control system that will help ensure an abundant supply of a new drug to treat malaria.

Scientists at the university's Research Institute of Pharmacy Science were tapped by the World Health Organization (WHO) to study the productivity of various lines of annual wormwood. Studies also explored the isolation of an antimalarial substance from the plant.

Annual wormwood has been used by the Chinese for 2,000 years to treat malaria. Researchers are currently testing a new, refined drug derived from the plant and hope that it can be used worldwide within the next 10 years. But for that to become a reality, wormwood must be produced on a large scale.

"Annual wormwood already grows throughout the United States and is often considered a weed," says Bryson. The woody-stemmed, cone-shaped plant reaches a height of 4 to 8 feet. Most U.S. farmers should be able to grow this potential cash crop.

Wormwood is harvested with a sickle-type machine similar to that used to cut kenaf and sugarcane. Once harvested, the cut plants must be protected from prolonged exposure to the sun's ultraviolet light, which breaks down the antimalarial substance—artemisinin.

Dried wormwood leaves are distilled to extract the artemisinin. To get about 1 gram of the substance, 1,000 grams or 2.2 pounds of the small leaves must be processed.

"At this rate, thousands of acres of wormwood would be needed to maintain a constant supply of the antimalarial drug," says Bryson. "That's why establishing a cropping system for annual wormwood is so important."

CHARLES BRYSON

About 2.2 pounds of the small leaves from the growing tip of the annual wormwood have to be processed to get 1 gram of the antimalarial substance, artemisinin.

But before it can be raised as a crop, farming methods, including weed control measures, must be developed for growers.

From 1985 through 1988, Bryson conducted tests to determine which herbicides would provide the best weed control with the least effect on the wormwood. Researchers first watched the effects of several herbicides on seedlings in growth chamber and greenhouse tests. Results of these experiments were used to narrow the number of herbicides used for field tests.

"Our greenhouse tests and field trials pinpointed three treatment systems that provided about 80- to 90-percent season-long weed control where seedling plants were established," says Bryson.

The top three systems all included postemergence applications of acifluorfen and fluazifop. But a pre-emergence treatment of chloramben provided the best weed control with the least noticeable crop injury, including effect on plant height, fresh weight, and the yield of artemisinin.

Preplant-incorporated trifluralin proved to be the second most effective system: the third included a pre-emergence application of metolachlor. "Looking just at weed control, without considering the other factors, the chloramben followed by the acifluorfen and fluazifop was again the best system," says Bryson. "The metolachlor treatment came in second, outperforming the trifluralin."

WHO estimates that 110 million people throughout the world contract malaria each year, causing 1 to 2 million deaths. A problem in the United States at the turn of the century, malaria was virtually wiped out by 1955, mainly through the use of DDT on mosquitos. Malaria victims were treated with quinine. But the disease is showing up again in the United States—mainly in Southern California—with 800 to 1,000 cases reported annually.

Malaria is caused by a parasite that is spread by the bite of the female anopheles mosquito. The parasites and mosquitos have apparently developed a resistance to current treatments.

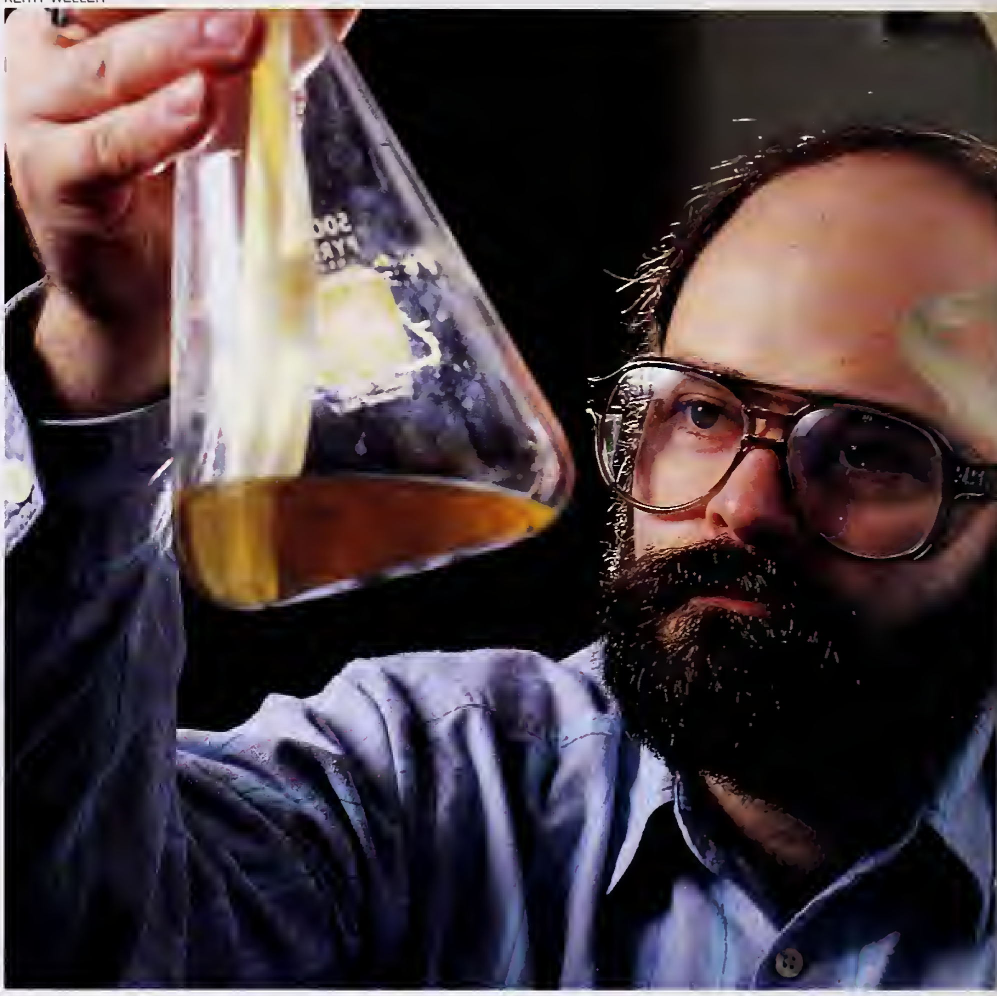
This resistance, coupled with a lack of drugs and doctors in countries where malaria outbreaks are the worst, is why new treatments are needed.

Tests of the drug arteether, made from artemisinin, are now being conducted in Europe. Approval by the U.S. Food and Drug Administration will be necessary before the drug is available in the United States.—By **Marcie Gerrietts,** ARS.

Charles T. Bryson is at the USDA-ARS Southern Weed Science Laboratory, P.O. Box 350, Stoneville, MS 38776. Phone (601) 686-5259. ◆

Fungal Diseases, Beware!

KEITH WELLER



Geneticist Thomas Hayman uses an ultrafiltration apparatus to concentrate toxin-containing culture for later analysis. (K-4672-1)

Two yeasts have been pinpointed that produce killer toxins that block the growth of other yeasts.

The toxins, more properly known as antifungal cytotoxic proteins, could be good news for agriculture and food industries looking for new weapons to fight pests, diseases, and dangerous microorganisms.

ARS geneticists G. Thomas Hayman, Paul L. Bolen, and colleagues identified the yeasts as strains of *Pichia inositovora* and *P. acaciae*. Hayman is with ARS' National Center for Agricultural Utilization Research in Peoria, Illinois. Bolen is now with International Flavors and Fragrances, Union Beach, New Jersey.

The toxin from *P. acaciae* tackles a large number of yeasts while that from *P. inositovora* seems more specific, singling out just a few to attack.

"We call them killer toxins," Bolen says, "though that may be somewhat of a misnomer. We know that they prevent sensitive yeasts from growing, but we've yet to determine how this inhibition occurs. It may be that the toxins aren't actively involved in the killing process."

Patent applications are under way for both toxins. *P. inositovora* and *P. acaciae* have been deposited as NRRL Y-18709 and NRRL Y-18665, respectively, in the ARS Patent Culture Collection. Commercial use of the technology will be available for licensing from USDA.

The concept of using yeast toxins against unwanted yeasts isn't new. A toxin from another yeast, *Kluyveromyces lactis,* has already been put to work in fermenting sake, or rice wine. That yeast's toxin—harmless to humans and animals—keeps out unwanted yeasts that may contaminate the process.

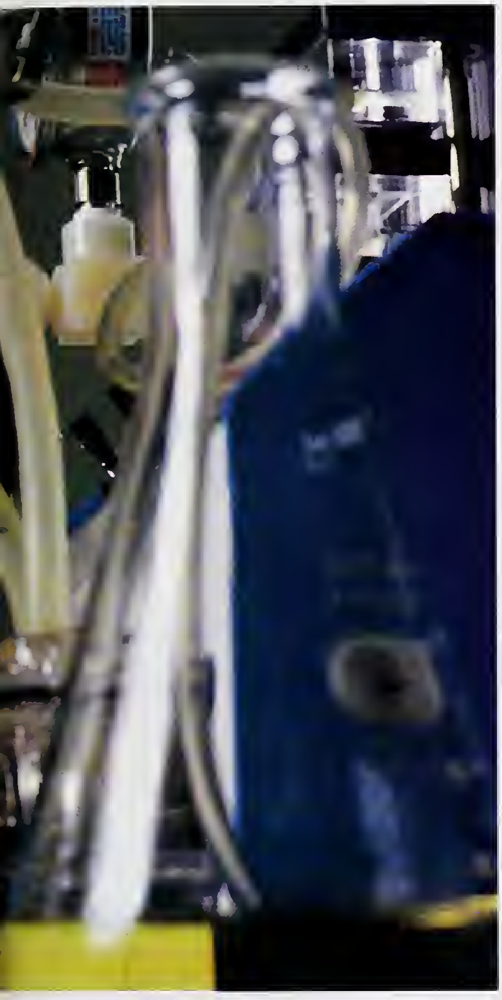
KEITH WELLER

Spelling out the appropriate word on a culture dish, patches of the yeast *Pichea acaciae* have inhibited the growth of a competing yeast (background) by releasing a cytotoxic protein. (K-4673-1)

Researchers think that the antifungal toxins from *P. acaciae* and *P. inositovora* are the products of genes carried on their linear plasmids—double-stranded pieces of DNA that are separate from the chromosome. Other genes appear to give each yeast immunity to its own toxin.

Linear plasmids occur also in molds and higher plants where they reside in mitochondria, the cells' power plants. But in these yeast strains, the plasmids are found in the inner portion of the yeast cell known as the cytoplasm.

Bolen and several colleagues at Peoria earlier discovered linear



BRUCE FRITZ

Microbiologist Cletus Kurtzman removes frozen yeast cultures from a liquid nitrogen freezer. (K-4383-4)

World's Largest Collection of Molds, Yeasts, and Bacteria

Some of science's most valuable tools are tucked away in a dark room on the third floor of the National Center for Agricultural Utilization Research (NCAUR) at Peoria, Illinois.

One of the world's largest microbial culture collections, the ARS Culture Collection contains more than 80,000 specimens of yeasts, molds, and bacteria of agricultural and industrial importance. Specific cultures related to microbiological inventions are deposited in conjunction with U.S. patent applications into the ARS Patent Culture Collection.

Since its establishment, the ARS Culture Collection has played a major role in the research on penicillin and other antibiotics, riboflavin, organic acids, xanthan and dextran gums, mycotoxins, and food fermentations.

Although the culture collection formally began when the Northern Regional Research Laboratory (now NCAUR) opened in 1940, Cletus Kurtzman, head of the collection, says that its origin can be traced back to 1904.

That's when Charles Thom joined USDA and began investigating Roquefort and Camembert cheeses. Thom and his assistant Margaret B. Church eventually acquired hundreds of mold cultures that became known as the Thom and Church collection.

The collection was deposited at the USDA facility at Peoria when Kenneth B. Raper, a colleague of Thom's, became head of the culture collection. Additional molds, bacteria, and yeasts were also deposited in the collection when it was formally established.

"Other cultures have been added to the collection as they've been acquired by scientists in USDA and the private sector," says Kurtzman. "In some cases, entire collections that might otherwise have been lost to science because of retirements or changes in research programs have been accessioned."

Most of the specimens in the collection are freeze-dried and stored in glass ampules. Specimens are stored at about 40°F and can be revived even after half a century. Some, though, are damaged by freeze-drying and are instead frozen in liquid nitrogen to keep them viable.

In the last decade, efforts have concentrated on finding microorganisms to use in the conversion of biomass to ethanol. Current and future work will be closely aligned with biotechnology.

"Scientists are using microorganisms from the collection in research to find natural ways to combat weeds, insects, and fungal infections," says Kurtzman. "We're also looking for ways to use microorganisms for the production of specialized organic compounds from low-cost agricultural products."

Cultures from the general collection are provided free of charge to anyone involved with research and development. More than 4,000 specimens are requested annually, says Kurtzman.

Culture strains deposited in the ARS Patent Culture Collection are maintained for at least the 17-year life of a U.S. patent and for 30 or more years when deposited under an international agreement such as the Budapest Treaty. The depositor may make the microorganism freely available on deposit or opt to wait until the patent has been issued.—By **Marcie Gerrietts,** ARS.

Cletus P. Kurtzman is at the USDA-ARS National Center for Agricultural Utilization Research, 1815 North University, Peoria, IL 61604. Phone (309) 685-4011, ext. 385.

KEITH WELLER

Using electrophoresis, geneticist Thomas Hayman separates yeast DNA fragments to show that toxin production is associated with certain linear plasmids. (K-4674-17)

plasmids in four strains of another yeast species. They were searching for plasmids that would aid the fermentation of xylose, a type of sugar.

Killer toxins from still other yeasts have been successful in treating fungal skin infections of experimental animals. The toxin from the *P. acaciae* strain might help fight mastitis, a fungal disease in cows; the most prevalent types of yeasts involved in this disease are sensitive to its toxin. However, the toxin's effect on mammals has yet to be determined.

In addition to producing toxins, these yeast strains and their linear plasmids promise to be important genetic engineering tools.

Scientists commonly use circular plasmids as vectors to move or shuttle genetic information from one organism to another. Pieces of linear plasmids can be inserted into a circular plasmid, enabling the circular plasmids to replicate more efficiently or in different host cells. Such plasmids would allow genes to be efficiently shuttled between different yeasts.

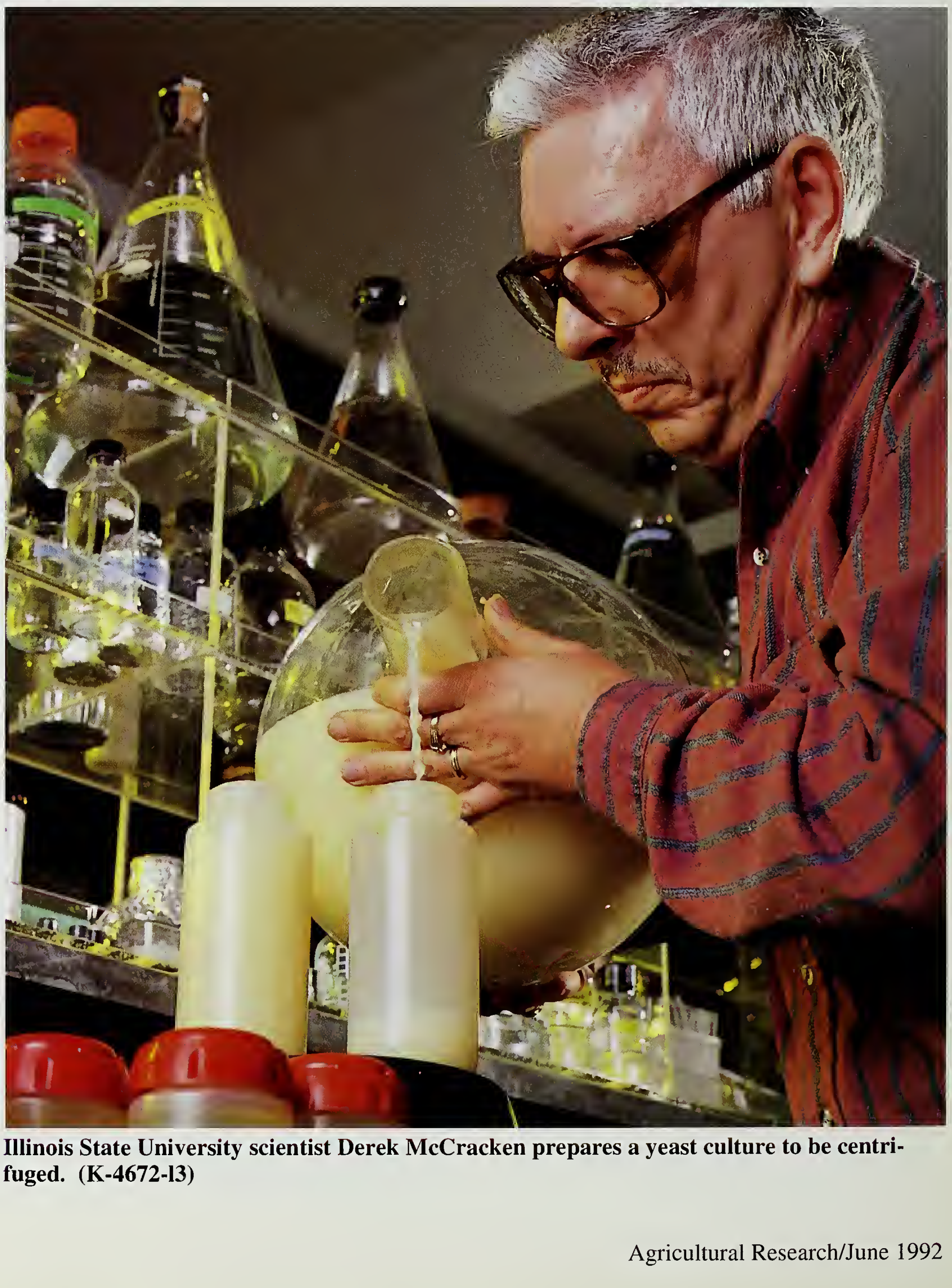
"Linear plasmids may themselves also work as vectors, though it may be more difficult to move them into other organisms," says Bolen.

"However, linear plasmids have a lot going for them. Unlike many circular plasmids, a single cell contains numerous copies of each linear plasmid, and they're extremely stable," Hayman says. "These two properties, high copy number and stability, may make them valuable genetic engineering tools."—By **Marcie Gerrietts,** ARS.

G. Thomas Hayman is in the USDA-ARS Biopolymer Research Unit, National Center for Agricultural Utilization Research, 1815 N. University, Peoria, IL 61604. Phone (309)685-4011.

For patent licensing information, contact the ARS Patent Coordinator, Room 403, Bldg. 005, BARC-West, 10300 Baltimore Ave., Beltsville, MD 20705-2350. Phone (301) 504-6786. ◆

KEITH WELLER



Illinois State University scientist Derek McCracken prepares a yeast culture to be centrifuged. (K-4672-13)

AGNOTES

Vitamin C Boosts Iron Absorption

Extra vitamin C at each meal can help women get more iron from vegetables, grains, and legumes.

In a 10-week study, women of childbearing age ate a diet very low in meat and high in plant foods from which iron is harder to absorb. During half of the experiment, they got a 500-milligram vitamin C supplement with each meal; during the other half they received a placebo.

The supplement increased neither blood hemoglobin nor iron reserves, but it did significantly boost the active thyroid hormone, T3.

Studies with rats have shown that iron deficiency reduces levels of T3, which regulates the body's metabolism. This supports findings in earlier studies showing that vitamin C can enhance iron absorption from plant foods.

Getting enough iron has long been a problem for younger women and may be becoming more so among those who cut back on consumption of red meat.

Adding a glass of orange or grapefruit juice or a serving of fresh strawberries or broccoli to meals could help overcome the problem.—By **Judy McBride,** ARS.

Janet R. Hunt is at the USDA-ARS Grand Forks Human Nutrition Research Center, P.O. Box 7166, University Station, Grand Forks, ND 58202. Phone (701) 795-8328. ◆

Shock Waves Map Chemical Shortcuts to Groundwater

Armed with a sledgehammer, a geologist is setting off shock waves in farm fields.

The shock waves are initially detected by sensitive instruments lowered down test wells in the fields.

With this measurement as a calibration, tiny instruments are then placed on the soil surface and more shock waves are generated. The recorded signals provide the data to map fracture zones beneath farm fields far beyond the well locations.

This type of instrumentation is normally associated with earthquake research, but the geologist in this case works for the Agricultural Research Service and his interest lies in mapping bedrock cracks through which groundwater can be contaminated.

James B. Urban, at University Park, Pennsylvania, says that "small quantities of nitrate and pesticides escaping the soil can be short-circuited by the cracks rather than being diluted, stored, or degraded in the soil. Groundwater beneath fractured rock with little soil cover can be especially vulnerable."

Water can carry chemicals more than a hundred times faster where cracks exist than where they do not, allowing little time for pesticide degradation.

"We'd like to give farmers an exact map of the rock fractures so they know what lies under each field," Urban says. "Then they can reconsider their chemical application or land use over trouble spots."

The mapping so far has shown that the significant fractures are most frequent above 30-foot depths. Shallow fracturing, especially at depths less than 30 feet, was found to be interconnected and continuous over large areas.

"Where this is the case, all wells should be encased and sealed above 30 feet."

In the shale formations common to this area, the interconnected fracturing extends no farther down than 75 to 110 feet.

"A farmer with this type of fracturing below 30 feet would be advised to check drinking water drawn from wells up to 110 feet deep," he says. "The deeper wells we've tested have been very low in any type of contamination."

Also, springs originating from deep fractures not connected to shallow fractures will most likely have the best water quality. "However," Urban says, "all springs should be tested before being used for drinking water."—By **Don Comis**, ARS.

James B. Urban is at the USDA-ARS Northeast Watershed Research Laboratory, 111 Research Bldg. A, University Park, PA 16802. Phone (814) 865-2048. ◆

History Sheds Fresh Light on Phytochrome

Forty years ago, a USDA research team in Beltsville, Maryland, made a startling find. It's recounted in detail in a new history of the decades-long search that led to the discovery of a bright-blue plant protein—phytochrome. "Pigment of the Imagination: A History of Phytochrome Research" was written by science writer Linda Sage and published in April 1992 by Academic Press, Inc.

Phytochrome remains one of USDA's most important—and intriguing—research discoveries. It acts as a unique molecular switch that controls germination, plant emergence from the soil, detection of neighboring plants, and time of flowering. [See *Agricultural Research,* September 1991, pp. 4-9.]

The 562-page book chronicles work from 1918 to 1991. Highlights include the first detection and extraction of phytochrome from seedlings in 1959 and a crucial experiment 7 years earlier. On April 9, 1952, ARS scientists first discovered the photoreversibility of the elusive pigment. Through this trait—never before observed in living organisms—the protein alters its form, back and forth, in response to red and far-red light.

The book contains extensive interviews with several members of the USDA phytochrome team including H. Marc Cathey, R. Jack Downs, Karl H. Norris, Albert A. Piringer, H. William Siegelman, and Vivian K. Toole, as well as with 100 other phytochrome scientists in the United States, Europe, and Japan.

The book may be ordered from Academic Press, 515 Westchester Drive, Campbell, CA 95008 (ISBN 0-12-614445-1).

Better Alfalfa for Harsh Climates

Alfalfa could become more important as a range plant in semi-arid regions, thanks to research at Fort Collins, Colorado.

There, an Agricultural Research Service scientist has succeeded in breeding greater seedling vigor into yellow-flowered alfalfa, a drought-tolerant cousin of the purple-flowered alfalfa that is the mainstay of animal production in the United States.

Purple-flowered alfalfa, *Medicago sativa* subsp. *sativa,* produces more forage and hay, but it can't survive the harsher environments found in grazing areas of the West. The yellow-flowered type, *M. sativa* subsp. *falcata,* is better adapted to such environments, but it is difficult to establish.

"It's a rare occasion when ranchers plant seed at the ideal time—just ahead of the perfect combination of weather conditions that the plant needs to become established," says plant geneticist Charley E. Townsend.

Since in many plant species, heavier seeds tend to give better seedling emergence, Townsend began breeding and selecting yellow-flowered alfalfa for heavier seed. The original plant material was provided by the ARS Plant Introduction Station in Ames, Iowa.

In 10 years, he has produced yellow-flowered types with seeds up to 20 percent heavier than the original stock. Seedling emergence of these seeds is equal to that of commercially available purple-flowered alfalfas.

"I believe further selection can improve seedling vigor even more, perhaps making alfalfa a better competitor with traditional western range grasses," says Townsend.—By **Dennis Senft,** ARS.

Charley E. Townsend is at the USDA-ARS Crops Research Laboratory, Grassland Resources Research Unit, Natural Resources Research Center, 1701 Center Ave., Fort Collins, CO 80526. Phone (303) 498-4231. ◆

New Pima Cotton: Longer Fibers for Finer Fabrics

After 12 years of research and testing, Pima S-7 is now available to cotton breeders and will be in growers' hands in time for the 1993 growing season.

Pima S-7 will eventually supersede Pima S-6, the current commercial variety, in many areas of the Pima Belt—from the San Joaquin Valley in California to south central Texas. S-7 was developed by Agricultural Research Service scientists in cooperation with the Arizona Agricultural Experiment Station at the University of Arizona in Tucson.

"Compared to S-6, S-7 matures earlier. This may reduce irrigation and pesticide applications, as well as allow earlier picking in areas that experience early fall rain. S-7 also has greater heat tolerance, which is important because high temperatures can cut yields," says retired ARS plant geneticist Edgar L. Turcotte at the Maricopa Agricultural Center, Maricopa, Arizona. Turcotte, the principal developer of Pima S-7, continues to work as a research collaborator.

Pima S-7 yielded 10 percent more fiber compared to S-6 in regional tests in Arizona, New Mexico, and Texas at low and intermediate elevations (sea level to 2,500 feet). Low and intermediate elevations make up 70 percent of the 240,000 acres planted with Pima cotton in the United States. In the remaining 30 percent of the acreage above 2,500 feet, Pima S-7 yields were similar to Pima S-6 yields.

"Pima S-7 has improved fiber, and when processed, the new variety gives stronger yarns with similar appearance and no more imperfections than earlier varieties," says ARS plant geneticist Richard G. Percy, who works with Turcotte.

Only about 2 percent of the total cotton grown in the United States is Pima, *Gossypium barbadense,* but it generates more income per pound for growers than upland cotton, *Gossypium hirsutum.* This is because Pima produces longer and stronger fibers. The longer the fiber, the finer the yarns that can be spun and the silkier the fabrics that can be manufactured.

These qualities are important to the high-fashion clothing market. Pima cotton is also used to make high-quality sheets, towels, and sewing thread.—By **Dennis Senft,** ARS.

Edgar L. Turcotte and Richard G. Percy are at the USDA-ARS Maricopa Agricultural Center, 37860 W. Smith-Enke Rd., Maricopa, AZ 85239. Phone (602) 379-4221. ◆

Climbing Mount Everest— An Energy Crisis

If you think running a marathon takes a lot of energy, try climbing the tallest mountain on earth. That's what former ARS chemist Robert D. Reynolds and lab technician M. Patricia Howard did in the spring of 1989 as part of a joint U.S.-Mexican expedition to Mt. Everest.

Along with many memories, they returned to the Beltsville Human Nutrition Research Center with hundreds of blood and urine samples and other data from the first major nutrition study at extreme altitudes. And the results are astounding.

A marathoner burns about 3,200 to 3,400 calories on race day, says Reynolds, who has run a few himself. The climbers, by contrast, burned an average 5,148 calories per day to maintain body temperature and perform their tasks at altitudes up to 29,000 feet above sea level. Four of them burned 6,000 to 8,000 calories daily.

He says the figures would be substantially higher on climbing days since the nine men and one woman rested for one or more days in between.

Howard and four others who remained at base camp to run the makeshift lab burned an average 3,286 calories per day. "That's equivalent to running a marathon every day," says Reynolds.

At 17,500 feet above sea level, base camp was 3,400 feet higher than the top of Pikes Peak...with no heat!

Despite the heavy energy toll, climbers ate only about half of the calories they burned on an average

daily basis, and lab technicians ate about 60 percent of what they burned.

Surprisingly, the team did not have anywhere near the up to 30 percent weight loss reported by other expeditions. Climbers averaged a 13 percent loss and technicians averaged 10.4 percent during their 9 weeks on the mountain.

This may in part be attributed to the fact that, to qualify for the expedition, each food item had to pass a tough test for palatability and ease of preparation. And many of the climbers' favorite foods were included in the 11,000 pounds carried to the mountain. "But," adds Reynolds, "there's no doubt that team members underreported their intakes. It happens in all food intake surveys."

Reynolds, who is now at the University of Illinois at Chicago, set out to learn what types of foods or diets may improve performance and reduce physical and mental trauma at such heights. All team members selected foods they felt gave them the most comfort or feeling of optimum performance from diets that alternated between high-fat/low-carbohydrate and low-fat/high-carbohydrate every 3 weeks.

Regardless of the type of diet offered, he says, the team chose foods that provided 30 percent of total calories as fat. Individually, fat intake ranged from 23 percent to 40 percent. Carbohydrates contributed 52 percent of the team's calories, ranging from 41 to 60 percent on an individual basis.

The fact that they chose a relatively low-fat diet was surprising because extra fat would have provided more calories, says Reynolds. "Yet it takes less oxygen to derive energy from carbohydrates than from fat," he notes. "The low oxygen at these altitudes may have triggered their desire for lower fat foods."—By **Judy McBride,** ARS.

For more information, contact Robert D. Reynolds at the Department of Nutrition and Medical Dietetics, University of Illinois at Chicago, 1919 West Taylor Street, Chicago, IL 60612. Phone (312) 996-1207. ◆

Nothing Worse Than Rusty Grass

Oregon-grown perennial ryegrass is one of the world's most widely used grasses. You've probably seen it in neighborhood parks, golf courses, and football stadiums. Sown in pastures, it provides food for sheep, beef, and dairy cattle across the nation.

But all is not idyllic with the growers who produce ryegrass seed. At the ARS National Forage Seed Production Research Center in Corvallis, Oregon, plant pathologist Ronald E. Welty is helping seed growers combat one of their worst problems—stem rust.

Caused by an airborne fungus, the disease changes leaf color from grassy green to rusty orange. The fungus forms pustules that each contain roughly 100,000 tiny fungal spores that drift to nearby plants and adjacent fields. In severely infected fields, seed yields can drop as much as 45 percent.

Stem rust also affects tall fescue, another widely planted grass for turf and forage.

Seed producers, who supply seed for nurseries, lawn and garden centers, and farmers, are the people who worry most about grass rusts. Cutting the grass—with a lawnmower or a hungry cow—reduces spore populations that spread the disease.

Last year, Oregon's perennial ryegrass crop was valued at over $54 million. Growers collectively spend about $2 million each time they spray fungicides against the rust—which is typically three times a year.

"Breeding new, rust-resistant varieties of Oregon ryegrass could save growers about $6 million each year," says Welty. "Also, there's a growing concern about the use of pesticides because of potential groundwater contamination; the length of time the chemicals remain in the soil; and their effects on other, nontarget organisms."

Farmers once burned straw stems—the residues left after grass seed is harvested—but air pollution regulations have recently restricted that practice. Stems without fungicide residues can be sold as roughage for livestock feed.

To find rust-resistant plants, Welty and colleagues exposed seedlings to the fungus in the greenhouse. Those that survived were again tested in the field. "Eventually, we hope to breed resistant plants that seed companies can cross with their own varieties," says Welty.—By **Julie Corliss,** ARS.

Ron Welty is with the USDA-ARS Forage Seed and Cereal Research Unit, 3540 S.W. Campus Way, Corvallis, OR 97330-7102. Phone (503) 750-8732. ◆

No Bones About It

Computer signals turn on high-pressure water jets to remove bone, fat, and gristle from beef chucks in a new, automated system for meat-packing plants.

ARS scientists use either electronic needles or optical or sonic sensors to detect unwanted meat parts and relay the information to the computer.

Water jets take out the rejected parts as they move down a conveyor belt. Result: a higher quality product for restructured beef cuts that are used primarily in institutional and convenience foods such as TV dinners.

This robotic processing system is five times faster than manually deboning and removing fat and gristle from beef chuck. It could lead to a savings of about $1 million a year for the U.S. meat industry.

Patent No. 4,970,757 has been awarded for this technology and two other applications have been made, Nos. 07,685,753 and 07,567,832.—By **Bruce Kinzel,** ARS.

For more information, contact James C. Craig, Jr., at the USDA-ARS Engineering Science Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118. Phone (215) 233-6589. ◆

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